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# Flavonoid Glycosides from Leaves of *Aucuba japonica* and *Helwingia japonica* (Cornaceae): Phytochemical Relationship with the Genus *Cornus*

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Seven flavonol and eleven flavone glycosides were isolated from the leaves of *Aucuba japonica* including var. *borealis* and *Helwingia japonica* including subsp. *liukiuensis*, respectively. They were identified or partially characterized as quercetin 3,7-di-*O*-glucoside, quercetin 3-*O*-sambubioside, quercetin 7-*O*-glucoside, quercetin 3-*O*-xyloside-7-*O*-glucoside, quercetin 3-*O*-xyloside-7-*O*-xylosylglucoside, kaempferol 3-*O*-xylosylglucoside, kaempferol 3-*O*-xyloside-7-*O*-glucoside (from *Aucuba japonica*), apigenin 7-*O*-glucoside, apigenin 7-*O*-diglucoside, apigenin 7-*O*-neohesperidoside, apigenin 7-*O*-xylosylglucoside, luteolin 7-*O*-glucoside, luteolin 7-*O*-diglucoside, luteolin 7-*O*-neohesperidoside, luteolin 7-*O*-xylosylglucoside, 6,8-di-*C*-glycosylapigenin, 6,8-di-*C*-glycosylluteolin and vitexin (from *Helwingia japonica*) by PC, FAB-MS, $^{1}$H- and $^{13}$H-NMR etc. The flavonoid characters were chemotaxonomically compared among *Aucuba*, *Helwingia* and *Cornus*. As the results, it was shown that *Helwingia* keeps aloof from *Cornus* and *Aucuba*.

*Aucuba japonica* Thunb. is endemic to Japan, but now cultivated as an ornamental plant in the world. On the other hand, *Helwingia japonica* (Thunb.) F.G. Dietrich including subsp. *liukiuensis* (Hatusima) Hara is distributed in Japan and rarely in China (Hara 1989). Iridoid, aucubin has been reported from *Aucuba japonica* as a secondary product (Bate-Smith et al. 1975).

The Cornaceae has chemotaxonomically been discussed in relation to various taxonomic treatments based on non-flavonoid substances, iridoids and ellagitannins (Jensen et al. 1975, Bate-Smith et al. 1975), but not studied by flavonoid components, which have already been used in many plants as chemotaxonomic markers (Harborne and Turner 1984).

As a part of chemotaxonomical studies of the Cornaceae, we have observed the flavonoid compositions of thirty-three *Cornus* taxa, and isolated and identified variously 3-*O*-glycosylated flavonols based on kaempferol, quercetin and rarely myricetin, i.e., glucoside, galactoside, rhamnoside, glucuronide, arabinoside, xyloside, rutinoside etc. (Iwashina and Hatta 1990, 1992, 1993, 1994).

In this paper, we describe the flavonoid compositions of *Aucuba japonica* including var. *borealis* and *Helwingia japonica* including subsp. *liukiuensis*, which are other genera in the Cornaceae, for chemotaxonomical elucidation of the systematics among *Cornus*, *Aucuba* and *Helwingia*, and compare the flavonoid characters of these species with those of *Cornus* species which we have previously reported.

## Materials and Methods

*Plant materials*

*Aucuba japonica* Thunb. var. *japonica* (TBG 49968) and var. *borealis* Miyabe & Kudo (TBG 7283) were cultivated in Tsukuba Botanical Garden, National Science Museum, Tsukuba, Ibaraki Pref. Two samples of *Helwingia japonica* (Thunb.) F.G. Dietrich were collected in Botanic Garden, Faculty of Agriculture, Hokkaido University, Sapporo, Hokkaido, and Hakone, Kanagawa Pref.

Voucher specimens of *H. japonica* subsp. *japonica* and subsp. *liukiuensis* were deposited in TNS.

*Isolation of flavonoids*

*Aucuba japonica* Fresh leaves (104 g) were extracted with MeOH, evaporated to dryness and dissolved in water. Crops were chromatographed on a polyamide column and eluted with 70% MeOH. The fractions containing same flavonoids were combined and applied to preparative paper chromatography (PPC) using solvent systems, BAW (n-BuOH/AcOH/$H_2O$ = 4:1:5, upper phase) and 15% AcOH. Flavonoids were purified by Sephadex LH-20 column (70% MeOH). Of isolated five flavonoids, four were obtained as pale yellow powders (**1** and **2**) or pure solution (**3** and **4**). It was proved by HPLC analysis that remaining flavonoid **5** was a mixture which consists of three flavonoids. A mixture was applied to PPC using solvent system, BEW (n-BuOH/EtOH/$H_2O$ = 4:1:2.2) and divided into three flavonoids (**5a**, **5b** and **5c**).

*Helwingia japonica* Fresh leaves (110 g) were extracted with MeOH and concentrated to aqueous residue *in vacuo*. The aq. extract was washed with petroleum ether and extracted with ethyl acetate (EtOAc). EtOAc layer which mainly contains flavonoids **6** and **7** was applied to polyamide column chromatography using 70% MeOH. Flavonoids **6** and **7** were separated by PPC using BAW and 15% AcOH. Finally, they were purified by Sephadex LH-20 column chromatography (70% MeOH) and obtained as pale yellow powders.

Aqueous residue was also applied to PPC using BAW and/or 15% AcOH. The separated seven flavonoids and some organic acids were purified by Sephadex LH-20 column (70% MeOH). Flavonoids **8**, **9**, **10** and **13** were obtained as pale yellow powders and **11**, **12** and **14** as pure solutions.

*High performance liquid chromatography (HPLC)*

HPLC separation was performed with JASCO HPLC systems. Multi channel UV-visible detector Multi-330 coupled with a computer was used to record chromatograms and UV spectra. A TSKgel ODS-80TM column (I.D. 4.6 × 150 mm) was used. Crude MeOH extracts or isolated flavonoid solutions were filtrated through Toyopak ODS M (Tosoh) and then Maisyoridisc, 0.45 $\mu$m (Tosoh), and eluted with $CH_3CN$-$H_2O$-$H_3PO_4$ (22:78:0.2, Sol. I) and (18:82:0.2, Sol. II). Detection was made at 200–350 nm and the flow-rate was 1.0 ml/min.

*Identification of flavonoids*

The flavonoid glycosides were identified by UV spectral analysis according to Mabry et al. (1970), fast atom bombardment mass spectra (FAB-MS) using nitrobenzyl alcohol (NBA), and $^1H$- and $^{13}C$-nuclear magnetic resonance ($^1H$- and $^{13}C$-NMR) spectra using dimethyl-sulfoxide-$d_6$ (DMSO-$d_6$) and direct PC and HPLC comparisons of original glycosides with authentic specimens.

Identification of their hydrolysates which were obtained by complete (12% aq. HCl, 100°C, 30 min) and partial acid hydrolysis (1.2% HCl:MeOH (1:1), 100°C, 10–40 min) was also performed by PC, TLC and HPLC comparisons with authentic specimens.

PC, UV, HPLC, FAB-MS, $^1$H- and $^{13}$C-NMR data were as follows.

Quercetin 3-*O*-xylosyl (1→2) glucoside (**1**). PC: Rf 0.42 (BAW), 0.56 (BEW), 0.54 (15%AcOH), 0.42 (5%AcOH); UV – dark purple, UV/$NH_3$ – dark yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 256, 265sh, 356; +NaOMe 272, 329, 407 (inc.); +$AlCl_3$ 275, 432; +$AlCl_3$/HCl 270, 297, 360, 400; +NaOAc 273, 324, 388; +NaOAc/$H_3BO_3$ 261, 377. HPLC: Retention time (Rt) 4.71 min (Sol. I). FAB-MS (NBA): $[M+H]^+$ at *m/z* 597, calcd. for $C_{26}H_{28}O_{16}$, $[M\text{–}xylosyl+H]^+$ at *m/z* 465, calcd. for $C_{21}H_{20}O_{12}$, $[M\text{–}xyloglucosyl+H]^+$ at *m/z* 303, calcd. for $C_{15}H_{10}O_7$. $^1$H-NMR (270 MHz, DMSO-$d_6$): $\delta$ 12.80 (1H, *s*, 5-OH), 7.79 (2H, *d*, *J* = 8.6 Hz, H-2', 6'), 6.96 (1H, *d*, *J* = 8.6 Hz, H-5'), 6.52 (1H, *s*, H-8), 6.31 (1H, *s*, H-6), 5.84 (1H, *d*, *J* = 6.9 Hz, glucosyl anomer), 5.53 (1H, *s*, xylosyl anomer), 5.1–3.6 (11H, *m*, glucosyl and xylosyl protons). $^{13}$C-NMR (67 MHz, DMSO-$d_6$): (aglycone) $\delta$ 155.4 (C-2), 133.1 (C-3), 177.5 (C-4), 161.3 (C-5), 98.7 (C-6), 164.1 (C-7), 93.5 (C-8), 156.3 (C-9), 104.6 (C-10), 122.0 (C-1'), 115.3 (C-2'), 145.0 (C-3'), 148.6 (C-4'), 116.1 (C-5'), 121.3 (C-6'); (glucosyl) $\delta$ 98.0 (C-1''), 81.9 (C-2''), 74.0 (C-3''), 69.5 (C-4''), 77.7 (C-5''), 60.7 (C-6''); (xylosyl) $\delta$ 104.0 (C-1'''), 76.2 (C-2'''), 76.9 (C-3'''), 69.6 (C-4'''), 65.7 (C-5''').

Quercetin 3-*O*-xylosyl (1→2) galactoside from the leaves of *Cornus capitata*. PC: Rf 0.51 (BAW), 0.53 (BEW), 0.58 (15%AcOH), 0.43 (5%AcOH); UV – dark purple, UV/$NH_3$ – yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 257, 264sh, 356; +NaOMe 272, 326, 405 (inc.); +$AlCl_3$ 275, 434; +$AlCl_3$/HCl 270, 297sh, 360, 401; +NaOAc 274, 326, 388; +NaOAc/$H_3BO_3$ 262, 375. FAB-MS (NBA): $[M+H]^+$ at *m/z* 597, calcd. for $C_{26}H_{28}O_{16}$, $[M\text{–}xylosyl+H]^+$ at *m/z* 465, calcd. for $C_{21}H_{20}O_{12}$, $[M\text{–}xylogalactosyl+H]^+$ at *m/z* 303, calcd. for $C_{15}H_{10}O_7$. $^1$H-NMR (270 MHz, DMSO-$d_6$): $\delta$ 12.91 (1H, *s*, 5-OH), 7.98 (2H, *d*, *J* = 8.6 Hz, H-2', 6'), 7.03 (1H, *d*, *J* = 8.6 Hz, H-5'), 6.61 (1H, *d*, *J* = 2.0 Hz, H-8), 6.40 (1H, *d*, *J* = 2.0 Hz, H-6), 5.91 (1H, *d*, *J* = 7.6 Hz, galactosyl anomer), 5.68 (1H, *s*, xylosyl anomer), 5.2–3.8 (11H, *m*, galactosyl and xylosyl protons). $^{13}$C-NMR (67 MHz, DMSO-$d_6$): (aglycone) $\delta$ 155.3 (C-2), 133.1 (C-3), 177.5 (C-4), 161.3 (C-5), 98.4 (C-6), 164.1 (C-7), 93.4 (C-8), 156.3 (C-9), 104.7 (C-10), 122.4 (C-1'), 115.3 (C-2'), 145.0 (C-3'), 148.6 (C-4'), 115.8 (C-5'), 121.3 (C-6'); (galactosyl) $\delta$ 98.6 (C-1''), 79.9 (C-2''), 73.7 (C-3''), 67.8 (C-4''), 74.0 (C-5''), 60.0 (C-6''); (xylosyl) $\delta$ 103.9 (C-1'''), 76.0 (C-2'''), 76.3 (C-3'''), 69.5 (C-4'''), 65.7 (C-5''').

Kaempferol 3-*O*-xylosylglucoside (**2**). PC: Rf 0.53 (BAW), 0.71 (BEW), 0.58 (15% AcOH), 0.44 (5%AcOH); UV – dark purple, UV/$NH_3$ – dark greenish yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 266, 349; +NaOMe 275, 325, 397 (inc.); +$AlCl_3$ 274, 304, 351, 394; +$AlCl_3$/HCl 275, 302, 346, 395; +NaOAc 274, 307, 383; +NaOAc/$H_3BO_3$ 267, 352. HPLC: Rt 7.14 min (Sol. I).

Quercetin 3,7-di-*O*-glucoside (**3**). PC: Rf 0.24 (BAW), 0.20 (BEW), 0.56 (15%AcOH), 0.43 (5%AcOH); UV – dark purple, UV/$NH_3$ – yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 256, 266sh, 359; +NaOMe 275, 415 (inc.); +$AlCl_3$ 274, 438; +$AlCl_3$/HCl 269, 295sh, 363, 398sh; +NaOAc 263, 405; +NaOAc/$H_3BO_3$ 261, 382. HPLC: Rt 2.24 min (Sol. I).

Kaempferol 3-*O*-xyloside-7-*O*-glucoside (**4**). PC: Rf 0.27 (BAW), 0.21 (BEW), 0.76 (15%AcOH), 0.73 (5%AcOH); UV – dark purple, UV/$NH_3$ – greenish yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 267, 349; +NaOMe 274, 386 (inc.); +$AlCl_3$ 269, 300, 351, 398sh; +$AlCl_3$/HCl 272, 298sh, 347, 395sh; +NaOAc and +NaOAc/$H_3BO_3$ 267, 352. HPLC: Rt 2.21 min (Sol. I).

Quercetin 7-*O*-glucoside (**5a**). PC: Rf 0.35 (BAW), 0.04 (15%AcOH); UV and UV/$NH_3$ – yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 256, 375; +NaOMe decomp.; +$AlCl_3$ 272, 460; +$AlCl_3$/HCl 266, 300sh, 365, 430; +NaOAc 259, 410; +NaOAc/

$H_3BO_3$ 260, 391. HPLC: Rt 9.00 min (Sol. I).

Quercetin 3-*O*-xyloside-7-*O*-xylosylglucoside? (**5b**). PC: Rf 0.23 (BAW), 0.11 (BEW), 0.69 (15%AcOH), 0.66 (5%AcOH); UV – dark purple, UV/$NH_3$ – dark yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 256, 266sh, 357; +NaOMe 273, 398 (inc.); +$AlCl_3$ 275, 437; +$AlCl_3$/HCl 270, 296sh, 365, 401; +NaOAc 262, 407; +NaOAc/$H_3BO_3$ 261, 381. HPLC: Rt 1.70 min (Sol. I).

Quercetin 3-*O*-xyloside-7-*O*-glucoside (**5c**). PC: Rf 0.23 (BAW), 0.16 (BEW), 0.69 (15%AcOH), 0.66 (5%AcOH); UV – dark purple, UV/$NH_3$ – dark yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 256, 266sh, 359; +NaOMe 273, 402 (inc.); +$AlCl_3$ 275, 439; +$AlCl_3$/HCl 270, 297sh, 365, 402; +NaOAc 262, 406; +NaOAc/$H_3BO_3$ 261, 383. HPLC: Rt 2.41 min (Sol. I).

Luteolin 7-*O*-glucoside (**6**). PC: Rf 0.31 (BAW and BEW), 0.08 (15%AcOH), 0.03 (5%AcOH); UV – dark purple, UV/$NH_3$ – bright yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 255, 265sh, 348; +NaOMe 266, 389 (inc.); +$AlCl_3$ 273, 427; +$AlCl_3$/HCl 275, 295, 358, 386; +NaOAc 260, 404; +NaOAc/$H_3BO_3$ 260, 373. HPLC: Rt 14.61 min (Sol. II).

Apigenin 7-*O*-glucoside (**7**). PC: Rf 0.50 (BAW and BEW), 0.16 (15%AcOH), 0.06 (5%AcOH); UV – dark purple, UV/$NH_3$ – yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 268, 333; +NaOMe 272, 378 (inc.); +$AlCl_3$ 275, 299, 348, 381; +$AlCl_3$/HCl 276, 298, 340, 381; +NaOAc 267, 387; +NaOAc/$H_3BO_3$ 268, 339. HPLC: Rt 25.89 min (Sol. II).

Luteolin 7-*O*-diglucoside (**8**). PC: Rf 0.16 (BAW), 0.24 (BEW), 0.20 (15%AcOH), 0.08 (5%AcOH); UV – dark purple, UV/$NH_3$ – bright yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 258, 263sh, 354; +NaOMe 270, 394 (inc.); +$AlCl_3$ 273, 424; +$AlCl_3$/HCl 273, 293sh, 355, 381sh; +NaOAc 259, 374; +NaOAc/$H_3BO_3$ 259, 369. HPLC: Rt 8.22 min (Sol. II).

Luteolin 7-*O*-neohesperidoside (**9a**) and luteolin 7-*O*-xylosylglucoside (**9b**). PC: Rf 0.29 (BAW), 0.35 (BEW), 0.22 (15%AcOH), 0.08 (5%AcOH); UV – dark purple, UV/$NH_3$ – bright yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 255, 264sh, 349; +NaOMe 266, 388 (inc.); +$AlCl_3$ 273, 426; +$AlCl_3$/HCl 274, 294, 359, 385; +NaOAc 259, 403; +NaOAc/$H_3BO_3$ 260, 373. HPLC: Rt 14.18 min (**9b**) and 18.57 min (**9a**) (Sol. II).

Apigenin 7-*O*-diglucoside (**10**). PC: Rf 0.31 (BAW), 0.35 (BEW), 0.32 (15%AcOH), 0.17 (5%AcOH); UV – dark purple, UV/$NH_3$ – yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 268, 334; +NaOMe 273, 378 (inc.); +$AlCl_3$ 275, 299, 349, 381; +$AlCl_3$/HCl 276, 299, 341, 380; +NaOAc 267, 389; +NaOAc/$H_3BO_3$ 268, 340. HPLC: Rt 13.83 min (Sol. II).

Apigenin 7-*O*-neohesperidoside (**11a**) and apigenin 7-*O*-xylosylglucoside (**11b**). PC: Rf 0.46 (BAW), 0.47 (BEW), 0.32 (15%AcOH), 0.17 (5%AcOH); UV – dark purple, UV/$NH_3$ – yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 268, 334; +NaOMe 271, 379 (inc.); +$AlCl_3$ 274, 299, 349, 377; +$AlCl_3$/HCl 277, 298, 341, 374sh; +NaOAc 268, 389; +NaOAc/$H_3BO_3$ 268, 339. HPLC: Rt 9.67 min (**11b**) and 12.33 min (**11a**) (Sol. I).

6,8-Di-*C*-glycosylluteolin (**12**). PC: Rf 0.10 (BAW), 0.14 (BEW), 0.33 (15%AcOH), 0.23 (5%AcOH); UV – dark purple, UV/$NH_3$ – dark yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 259, 272, 348; +NaOMe 271, 337sh, 411 (inc.); +$AlCl_3$ 278, 425; +$AlCl_3$/HCl 279, 297sh, 357, 383sh; +NaOAc 281, 401; +NaOAc/$H_3BO_3$ 268, 378, 425sh. HPLC: Rt 3.02 min (Sol. II).

6,8-Di-*C*-glycosylapigenin (**13**). PC: Rf 0.18 (BAW), 0.23 (BEW), 0.44 (15%AcOH), 0.35 (5%AcOH); UV – dark purple, UV/$NH_3$ – dark yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 273, 332; +NaOMe 283, 334, 400 (inc.); +$AlCl_3$ 279, 305, 352, 382sh; +$AlCl_3$/HCl 280, 304, 345, 378sh; +NaOAc 282, 393; +NaOAc/$H_3BO_3$ 285, 322, 405sh. HPLC: Rt 3.96 min (Sol. II).

8-*C*-glucosylapigenin (vitexin, **14**). PC: Rf 0.40 (BAW), 0.38 (BEW), 0.17 (15%AcOH), 0.09 (5%AcOH); UV – dark purple, UV/$NH_3$ – dark yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 269, 336; +NaOMe 279, 332, 394 (inc.); +$AlCl_3$ 276, 303, 350, 389; +$AlCl_3$/HCl 277, 302, 343, 379; +NaOAc 278, 307sh, 386; +NaOAc/$H_3BO_3$ 269, 348. HPLC: Rt 10.14 min (Sol. II).

Chlorogenic acid. PC: Rf 0.33 (BAW), 0.30 (BEW), 0.71 (15%AcOH), 0.69 (5%AcOH); UV – dark blue, UV/$NH_3$ – dark green. UV: $\lambda_{max}^{MeOH}$ (nm) 280, 323; +NaOMe 293sh, 379 (inc.); +$AlCl_3$ 267, 314, 354sh; +$AlCl_3$/HCl 281, 321. HPLC: Rt 2.70 min (Sol. I).

## Results

*Flavonoids from* Aucuba japonica

Seven flavonoid glycosides were isolated from the leaves of *A. japonica.* In this experiment, the flavonoid profile of *A. japonica* var. *borealis* was also surveyed, but it could not be essentially distinguished with that of *A. japonica* var. *japonica.*

Major flavonoid **1** liberated quercetin, glucose and xylose by complete acid hydrolysis. UV spectra of original glycoside showed the presence of free 5-, 7-, 3'- and 4'-hydroxyl and a substituted 3-hydroxyl group. FAB-MS exhibited $[M+H]^+$ at *m/z* 597 and $[M\text{–}xylosyl+H]^+$ at *m/z* 465 showing the presence of each 1 mol quercetin, glucose and xylose, and direct attachment of glucose to quercetin. $^1$H-NMR spectra of original glycoside indicated five aromatic protons (H-6, -8, -2', -5' and -6') and *β*-D-glucopyranosyl anomeric proton (δ 5.84, *d*, $J = 6.9$ Hz). Since xylosyl anomeric proton, moreover, appeared as a singlet (δ 5.53), xylose was regarded as *β*-D-xylofuranosyl but not xylopyranosyl (Markham and Geiger 1994). It was shown that xylose was on 2-OH of 3-*O*-glucosyl group, by $^{13}$C-NMR comparisons of the glycoside with those of isorhamnetin 3-*O*-rhamnosyl (1→2) glucoside (Agrawal and Bansal 1989), quercetin 7,3'-dimethyl ether 3-*O*-rhamnosyl (1→2) glucoside (Itokawa et al. 1981) and isorhamnetin 3-*O*-rhamnosyl (1→6) glucoside (Markham et al. 1978).

Thus, flavonoid **1** was identified as quercetin 3-*O*-*β*-D-xylofuranosyl (1→2)-*O*-*β*-D-glucopyranoside.

Quercetin 3-*O*-xylosylglucoside have been isolated as 3-*O*-*β*-D-xylopyranosyl (1→2)-*O*-*β*-D-glucopyranoside, i.e., 3-*O*-sambubioside from the flowers of *Hibiscus mutabilis* L. f. *versicolor* Makino (Malvaceae) (Ishikura 1982) and the leaves of *Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq. var. *giraldii* (Diels) Voroshilov (Actinidiaceae) (Webby 1991). In this survey, xylose was reported as xylofuranose but not xylopyranose, for the first time.

In relation to above flavonol glycoside, we have isolated quercetin 3-*O*-xylosylgalactoside from the leaves of *Cornus capitata* Wall. (Iwashina and Hatta 1993). In this survey, the flavonol glycoside was also proved to be quercetin 3-*O*-*β*-D-xylofuranosyl (1→2)-*O*-*β*-D-galactopyranoside based on PC, FAB-MS, $^1$H- and $^{13}$C-NMR data (see Materials and Methods). Quercetin 3-*O*-xylosylgalactoside have been isolated as 3-*O*-*β*-D-xylopyranosyl (1→2)-*O*-*β*-D-galactopyranoside from the leaves of *Armoracia rusticana* G. M. & Sch. (Cruciferae) (Larsen et al. 1982).

UV spectra of flavonoid **2** showed the presence of free 5-, 7- and 4'-hydroxyl and a substituted 3-hydroxyl group. Kaempferol, glucose and xylose were liberated by complete acid hydrolysis. From the results desribed above, flavonoid **2** was identified as kaempferol 3-*O*-xylosylglucoside, probabry 3-*O*-sambubioside as flavonoid **1**.

Minor flavonoid **3** produced quercetin and glucose, and quercetin 3-*O*-glucoside and 7-*O*-glucoside by complete and partial acid hydrolysis, respectively. In addition, UV spectra of original glycoside showed the presence of free 5,3',4'-triOH and substituted 3,7-diOH.

Thus, flavonoid **3** was identified as quercetin 3,7-di-*O*-glucoside.

Flavonoid **4** showed the lower Rf values in alcoholic solvent systems, BAW and BEW, and higher in aqueous ones, 15%AcOH and

5%AcOH. Kaempferol, glucose and xylose were liberated by complete acid hydrolysis. UV spectra of original glycoside showed the presence of free 5- and 4'-hydroxyl and substituted 3- and 7-hydroxyl groups. Since the flavonoid produced kaempferol 7-*O*-glucoside and a trace of unknown intermediate, probabry labile kaempferol 3-*O*-xyloside, which were monitored by HPLC, by partial acid hydrolysis, flavonoid **4** is regarded as kaempferol 3-*O*-xyloside-7-*O*-glucoside. Kaempferol 3-*O*-xyloside-7-*O*-glucoside has been found in *Zinnia elegans* Jacq. (Compositae) (Harborne and Williams 1988).

Flavonoid **5a** was visuarized to yellow by UV lamp without exposure to fuming ammonia, showing to be a flavonol glycoside or an aglycone having free 3- and 4'-hydroxyl groups. Quercetin and glucose were liberated as the hydrolysates. UV spectra of original glycoside showed the presence of free 3,5,3',4'-tetraOH.

From the results described above, flavonoid **5a** was identified as quercetin 7-*O*-glucoside.

Complete acid hydrolysis of flavonoids **5b** and **5c** gave quercetin, glucose and xylose. UV spectra of original glycosides showed the presence of free 5-, 3'- and 4'-hydroxyl and substituted 3- and 7-hydroxyl groups. Partial acid hydrolysis of **5c** gave quercetin 7-*O*-glucoside (**5a**) and a trace of quercetin 3-*O*-xyloside as intermediates. On the other hand, partial acid hydrolysis of **5b** gave **5c** and **5a** as intermediates.

From the results described above, **5c** was identified as quercetin 3-*O*-xyloside-7-*O*-glucoside and **5b** was presumed as quercetin 3-*O*-xyloside-7-*O*-xylosylglucoside.

### *Flavonoids from* Helwingia japonica

Two samples from The Botanic Garden, Hokkaido Univ. and Hakone, Kanagawa Pref. of *H. japonica* subsp. *japonica* and one from Mt. Nago, Okinawa Pref. of *H. japonica* subsp. *liukiuensis* were used as plant materials. Their flavonoid compositions which were surveyed by 2D-PC and HPLC were essentially the same.

Eleven flavonoids were isolated from the leaves.

Major flavonoids **6** and **7** were shown to possess of free 5,3',4'-triOH and 5,4'-diOH, respectively, by UV spectra. The aglycones and sugars obtained by acid hydrolysis were identified as luteolin and glucose (**6**), and apigenin and glucose (**7**) by direct PC comparisons with authentic specimens, respectively.

Finally, flavonoids **6** and **7** were identified as luteolin 7-*O*-glucoside (**6**) and apigenin 7-*O*-glucoside (**7**) by direct PC and HPLC comparisons with authentic specimens.

It was shown by UV spectra and complete acid hydrolysis that flavonoids **8** and **10** were also luteolin and apigenin glycosides which attached glucose to each 7-hydroxyl group. Each one intermediate which was obtained by partial acid hydrolysis of flavonoids **8** and **10** was identified as 7-*O*-monoglucosides of luteolin and apigenin.

Accordingly, flavonoids **8** and **10** were regarded as luteolin 7-*O*-diglucoside and apigenin 7-*O*-diglucoside, respectively.

Flavonoid **9** appeared as single spot on the paper chromatogram, but it divided into two peaks (**9a** and **9b**) by HPLC analysis. Complete acid hydrolysis of the mixture produced luteolin, glucose, rhamnose and xylose. Luteolin 7-*O*-glucoside was obtained as an intermediate by partial acid hydrolysis of the mixture. Since the presence of free 5-, 3'- and 4'-hydroxyl groups was shown by UV spectral analysis of original glycoside, it is clear that sugars are on 7-position of luteolin. Of two peaks on HPLC, retention time of **9a** agreed with that of authentic luteolin 7-*O*-rhamnosyl (1→2) glucoside (luteolin 7-*O*-neohesperidoside). Accordingly, another one (**9b**) is regarded as luteolin 7-*O*-xylosylglucoside.

Flavonoid **11** also appeared as single spot on paper chromatogram, but it was divided into two peaks (**11a** and **11b**) by HPLC separation. Apigenin, glucose, rhamnose and xylose, and apigenin 7-*O*-glucoside were obtained by complete and partial acid hydrolysis, respectively. UV spectra of the mixture showed the presence of free 5,4'-diOH and a substituted 7-OH. Since retention time of peak **11a** agreed with that of authentic apigenin 7-*O*-neohesperidoside (rhoifolin), another one (**11b**) was characterized as apigenin 7-*O*-xylosylglucoside.

Flavonoids **12**, **13** and **14** could not be hydrolyzed by hot acid treatment showing that the compounds are *C*-glycosylflavonoids. UV spectra of **13** and **14** showed the presence of free 5-, 7- and 4'-hydroxyl groups (apigenin type). On the other hand, UV spectra of **12** exhibited the presence of free 5,7,3',4'-tetraOH (luteolin type). Hot acid treatment of **14** gave another spot (an isomer of **14**) on the chromatogram in addition to original one, which was caused by Wessely-Moser rearrangement (Markham 1982). On the other hand, no rearrangement occurred in **12** and **13**. Thus, it was shown that **12** and **13** are 6,8-di-*C*-glycosylflavones and **14** is 6- or 8-mono-*C*-glycosylflavone.

From the results described above, flavonoids **12** and **13** were characterized as 6,8-di-*C*-glycosylluteolin and 6,8-di-*C*-glycosylapigenin, respectively. However, retention times of **12** and **13** did not agree with those of 6,8-di-*C*-glucosylluteolin (lucenin-2) and 6,8-di-*C*-glucosylapigenin (vicenin-2).

Flavonoid **14** was identified as 8-*C*-glucosylapigenin (vitexin) by PC and HPLC comparison with authentic specimen.

In addition to these flavonoid glycosides, some phenolic compounds were isolated from the leaves of *Helwingia japonica*. One of them was identified as chlorogenic acid (3-caffeoylquinic acid) by characterization of hydrolysates, UV spectra, and PC and HPLC comparison with authentic specimen. Other components were characterized as chlorogenic acid derivative (one kind) and caffeoyl sugar esters (four kinds).

## Discussion

The flavonoids of *Aucuba japonica* including var. *borealis* and *Helwingia japonica* including subsp. *liukiuensis* were reported in this paper for the first time. In spite of both genera belonging to the family Cornaceae, their flavonoid characters were clearly different, i.e., the former flavonoids consist of flavonol 3-*O*-di- and 3,7-di-*O*-glycosides based on kaempferol and quercetin, while the latter ones are constituted with flavone 7-*O*-glycosides and *C*-glycosyl derivatives based on apigenin and luteolin. We have observed the flavonoids in the leaves of thirty-three *Cornus* taxa (Iwashina and Hatta 1990, 1992, 1993, 1994). Their major flavonoids were various flavonol 3-*O*-monoglycosides based on kaempferol and quercetin, i.e., glucoside, galactoside, glucuronide, rhamnoside, xyloside and arabinoside, in company with minor myricetin 3-*O*-rhamnoside and flavonol 3-*O*-diglycosides such as quercetin 3-*O*-rutinoside, kaempferol 3-*O*-rutinoside, quercetin 3-*O*-xylosylgalactoside etc. (Table 1). Flavonoid characters of *Cornus* species were similar to those of *Aucuba* species with regard to occurrence of flavonols alone. However, major flavonol 3-*O*-monoglycosides which were found from all *Cornus* species examined (Iwashina and Hatta 1994) were completely absent from *Aucuba japonica*. Instead, flavonol 3,7-di-*O*-glycosides such as kaempferol and quercetin 3-*O*-xyloside-7-*O*-glucosides were found from *Aucuba japonica* together with 3-*O*-xylosylglucosides as major components. In addition, two anthocyanins, 3-*O*-xylosylglucosides of both pelargonidin and cyanidin have been iso-

Table 1. The flavonoid glycosides isolated from *Cornus*, *Aucuba* and *Helwingia* species

| Genera | | Glycosides |
|---|---|---|
| *Cornus* | kaempferol | 3-glucoside(12), 3-galactoside(13), 3-glucuronide(5), 3-diglucoside(1), 3-rutinoside(2), 3-xylogalactoside(2), 3,7-dirhamnoside(1), 3-glucoside-7-rhamnoside(1), 3-galactoside-7-rhamnoside(1) |
| | quercetin | 3-glucoside(30), 3-galactoside(14), 3-glucuronide(11), 3-rhamnoside(9), 3-arabinoside(5), 3-xyloside(5), 3-diglucoside(2), 3-rutinoside(15), 3-xyloglucoside(1), 3-xylogalactoside(4), 3-glucoside-7-rhamnoside(1), 3-galactoside-7-rhamnoside(1) |
| | myricetin | 3-rhamnoside(5) |
| *Aucuba* | kaempferol | 3-xyloglucoside, 3-xyloside-7-glucoside |
| | quercetin | 7-glucoside, 3-xyloglucoside, 3,7-diglucoside, 3-xyloside-7-glucoside, 3-xyloside-7-xyloglucoside |
| *Helwingia* | apigenin | 7-glucoside, 7-diglucoside, 7-neohesperidoside, 7-xyloglucoside, 8-*C*-glucosyl and 6,8-di-*C*-glycosyl derivatives |
| | luteolin | 7-glucoside, 7-diglucoside, 7-neohesperidoside, 7-xyloglucoside, 6,8-di-*C*-glycosyl derivative |

( ) = Numbers of species which the glycoside was found from examined 33 *Cornus* species (Iwashina and Hatta 1990, 1992, 1993, 1994).

Table 2. Comparison of flavonoid characters among the genera *Cornus*, *Aucuba* and *Helwingia*

| Flavonoids | *Cornus* | *Aucuba* | *Helwingia* |
|---|---|---|---|
| Flavonols | | | |
| myricetin | + | | |
| 3-monoglycosides | + | | |
| 3-diglycosides | + | + | |
| 3,7-glycosides | + | + | |
| 7-glycoside | | + | |
| Flavones | | | |
| 7-glycosides | | | + |
| 7-diglycosides | | | + |
| *C*-glycosyl derivatives | | | + |

lated from the fruits of *A. japonica* (Ishikura 1971) showing that the flavonoid 3,7-diglycosides and 3-*O*-xylosylglycosides are principal chemical character of the species or genus. It is noteworthy that both flavonoid characters were reported from Chinese *Cornus* species, *C. multinervosa* (Pojark.) Q. Y. Xiang belonging to subgenus Benthamia (Iwashina and Hatta 1994).

On the other hand, major flavonoids of *Helwingia* were flavone 7-*O*-glycosides together with minor *C*-glycosides, and their chemical characters were clearly distinguishable from those of *Cornus*, *Aucuba* and also *Mastixia* (Iwashina and Hatta unpublished data) containing flavonol glycosides as major compounds (Table 2). Bate-Smith et al. (1975) have also proposed to raise the genus *Helwingia* to family rank within Cornales due to the absence of any tannins and iridoids (the presence in *Cornus*, *Aucuba* and *Mastixia*). The facts described above show that *Helwingia* phytochemically keeps aloof from *Cornus*, *Aucuba* and *Mastixia*. Moreover, they were also supported by *rbc*L sequence data (Xiang et al. 1993).

In delineating the position of *Cornus*, characterization of flavonoids in other Cornaceae genera and related families, Alangiaceae, Nyssaceae and Davidiaceae are needed and it is now in progress by us.

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岩科　司，亀之園浩治，八田洋章：**アオキおよびハナイカダ（ミズキ科）の葉に含まれるフラボノイド配糖体：ミズキ属植物との植物化学的関係**

アオキ，ヒメアオキ，ハナイカダおよびリュウキュウハナイカダの葉に含まれるフラボノイド配糖体が今回初めて報告された．アオキとその変種のヒメアオキは同一のフラボノイドを有しており，それらはquercetinの3-*O*-sambubioside，7-*O*-glucoside，3, 7-di-*O*-glucoside，3-*O*-xyloside-7-*O*-glucosideおよび3-*O*-xyloside-7-*O*-xylosylglucoside，kaempferolの3-*O*-xylosylglucosideおよび3-*O*-xyloside-7-*O*-glucosideと同定された．

一方，ハナイカダとその亜種のリュウキュウハナイカダも同一のフラボノイドを有し，apigeninとluteolinの7-*O*-glucoside，7-*O*-diglucoside，7-*O*-neohesperidoside，7-*O*-xylosylglucosideおよび6, 8-di-*C*-glycoside，およびvitexinと同定された．

これらのフラボノイドを，以前に報告したミズキ属植物のもの（岩科・八田 1990，Iwashina and Hatta 1992，1993，1994）と比較すると，アオキとミズキ属植物のフラボノイドはいずれもフラボノール配糖体である点で一致するが，調査されたミズキ属すべての植物の主要配糖体が一般的なフラボノール3-*O*-モノ配糖体であるのに対して，アオキではほとんどがキシロースを結合した3-*O*-ジ配糖体や3, 7-*O*-配糖体であることで区別された．

また，ハナイカダから分離されたフラボノイドはすべてがフラボンの*O*-および*C*-配糖体であり，ミズキ属植物やアオキとは明らかに異なっており，この属がミズキ属やアオキ属とはかなり離れた位置にあることを示唆した．

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